Differential expression of the pro-natriuretic peptide convertases corin and furin in experimental heart failure and atrial fibrosis


Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota

Submitted 23 May 2012; accepted in final form 12 November 2012

Ichiki T, Boerrigter G, Huntley BK, Sangaralingham SJ, McKie PM, Harty GJ, Harders GE, Burnett JC Jr. Differential expression of the pro-natriuretic peptide convertases corin and furin in experimental heart failure and atrial fibrosis. Am J Physiol Regul Integr Comp Physiol 304: R102–R109, 2013. First published November 14, 2012; doi:10.1152/ajpregu.00233.2012.—In heart failure (HF), the cardiac hormone natriuretic peptides (NPs) atrial (ANP), B-type (BNP), and C-type (CNP) play a key role to protect cardiac remodeling. The proprotein convertases corin and furin process their respective pro-NPs into active NPs. Here we define in a canine model of HF furin and corin gene and protein expression in normal and failing left atrium (LA) or ventricle (LV) testing the hypothesis that the NP proproteins convertases production is altered in experimental HF. Experimental canine HF was produced by rapid right ventricular pacing for 10 days. NPs, furin, and corin mRNA expression were determined by quantitative RT-PCR. Protein concentration or expression was determined by immunostaining, radioimmunooassay, or Western blot. Furin and corin proteins were present in normal canine LA and LV myocardium and vasculature and in smooth muscle cells. In normal canines, expression of NPs was dominant in the atrium compared with the ventricle. In experimental early stage HF characterized with marked atrial fibrosis, ANP, BNP, and CNP mRNA, and protein concentrations were higher in HF LA but not HF LV compared with normals. In LA, corin mRNA and protein expressions in HF were lower, whereas furin mRNA and protein expressions were higher than normals. NPs and furin expressions were augmented in the atrium in experimental early stage HF and, conversely, corin mRNA and protein expressions were decreased with atrial remodeling. Selective changes of these NP convertases may have significance in the regulation of pro-NP processing and atrial remodeling in early stage HF.

A HALLMARK OF HEART FAILURE is the increased synthesis of the cardiac natriuretic peptides, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), which are of myocardial cell origin. After binding to the guanylyl cyclase (C)-A receptor, they function to regulate cardiorenal function and to modulate myocardial structure and function by direct actions on cardiac myocytes and fibroblasts (17, 19, 20, 30, 45). C-type NP (CNP), which is principally of endothelial cell origin, plays an important role in vascular and endothelial function via a separate GC-B receptor (43). Unlike ANP or BNP, circulating CNP is unchanged or minimally elevated in human heart failure. In the presence of aging, which is a hallmark of heart failure, CNP may actually be reduced in the circulation (36, 38, 47).

Importantly, evidence suggests impaired processing in heart failure of at least one natriuretic peptide, which is the molecular precursor of BNP such that the principal circulating molecular form of BNP is the biologically inactive pro-BNP (16, 18). Indirect measurement of pro-ANP also suggests possible impaired processing in human heart failure (22). The processing of CNP in heart failure remains undefined. The processing of the natriuretic peptides in the heart most likely occurs under normal conditions in the atria where ANP and BNP gene expression are robust, while essentially absent in the ventricle. This underscores the important role for the cardiac atria and its major role as an endocrine organ as originally put forth by de Bold et al. (11).

Fundamental to the activation of the natriuretic peptides as with other peptides is the posttranslational cleavage of an inactive precursor protein (i.e., the prohormone) into a mature biologically active peptide. Specifically, ANP, BNP, and CNP are derived from their respective prohormones pro-ANP, pro-BNP, and pro-CNP. Studies support the conclusion that two proprotein convertases, furin and corin, mediate pro-NP processing. Corin, a transmembrane serine protease, exists in the heart as well as the kidney and the circulation, and converts pro-ANP to ANP (23, 49). Corin-deficient mice are characterized by decreased pro-ANP conversion to ANP, increased blood pressure, and cardiac hypertrophy (7). Furin, an intracellular endoprotease, which is enriched in the Golgi apparatus, functions to cleave proproteins into their mature and active forms. Wu et al. (48) reported that furin converts pro-CNP into CNP in furin expressed cells but not in furin-deficient LoVo cells. Semenov et al. (40) reported both corin and furin cleave pro-BNP into BNP fragments in vitro. Taken together, pro-ANP is cleaved by corin, pro-CNP is cleaved by furin, and pro-BNP is cleaved by both furin and corin. To date, information is incomplete regarding the regulation of both corin and furin in the failing heart, especially during the early stages of heart failure. Indeed, it is important to fully understand the presence and regulation of expression of these two key natriuretic peptide convertases both in our understanding of the biology of these enzymes and in the control of the natriuretic peptides in heart failure.

Therefore, the objective of the current study was to first define the atrial and ventricular gene expression and protein concentrations of the three natriuretic peptides in a model of experimental heart failure, which may more reflect the early evolving stages of human heart failure. Use of such a model permits complete tissue harvesting of the LA and LV, together with carefully characterization of cardiovascular hemodynamics, all in the absence of heart failure medications, which may have secondary actions of hemodynamics or tissue function or structure. Second, based on our findings of the key role for the atria and not the ventricles in this early stage heart failure, we then defined the presence of corin and furin in the left atrial...
myocardium of normal hearts by immunohistochemistry. Finally, we defined the mRNA and protein expression of corin and furin in the LA in normal and failing canine hearts. We specifically tested the hypothesis that experimental heart failure, with marked increases in atrial pressure and atrial fibrosis, would be characterized by a reduction in atrial corin mRNA and protein expression in association with marked increases in ANP and BNP production, while furin would be unaffected in association with minimal increases in CNP production or tissue CNP levels in heart failure.

MATERIALS AND METHODS

This study was in accordance with the Animal Welfare Act, and was approved by the Mayo Clinic Animal Care and Use Committee. All human experimental protocols used in the current study were approved by the Institutional Review Board at Mayo Clinic.

Normal and heart failure canines. Studies were conducted in two groups of male mongrel dogs (weight, 20 to 28 kg), normal control and pacing-induced experimental heart failure. Severe heart failure was induced in dogs by rapid right ventricular pacing at 240 beats/min as previously described and characterized (5, 8, 10). In brief, all heart failure dogs underwent implantation of a programmable cardiac pacemaker (Medtronic, MN). After a 14-day postoperative recovery period, the pacemaker was turned on at 240 beats/min. On day 11 of rapid ventricular pacing, the experiment was carried out while the dog was anesthetized with pentobarbital sodium (15 mg/kg), intubated, and mechanically ventilated with supplemental oxygen (Harvard respirator) at 12 cycles/min to determine hemodynamic parameters and to collect blood samples for humoral parameters. The left femoral artery and vein were cannulated for measuring hemodynamic data, including atrial blood pressure, and then a balloon-tipped thermolysis catheter (American Edwards Laboratory) was inserted to measure right atrial pressure (RA), cardiac output (CO), and pulmonary capillary wedge pressure (PCWP). After the experiments, the tissue was harvested.

Histological analysis for fibrosis by picrosirius red staining. Fixed canine LA and LV tissues were dehydrated, embedded in paraffin, and sectioned at a thickness of 4 µm. Collagen and extent of fibrosis was performed using picrosirius red staining. An Axioplan II KS 400 microscope (Carl Zeiss) was used to capture at least four randomly selected images from each slide using a 40 objective, and KS 400 software was utilized to determine fibrotic area as a percentage of total tissue area.

Cell culture. Human aortic smooth muscle cells (HASMCs) (Lonza, Walkersville, MD) were cultured in uncoated plastic and maintained in smooth muscle cells media with supplements (Lonza) at 37°C in 5% CO2-95% air in a humidified atmosphere according to manufacturer’s instruction. HASMCs at passages 4 were seeded on 8 chamber polystyrene tissue culture glass slides (BD Falcon) and used for immunocytochemistry.

Immunohistochemistry and immunocytochemistry for corin and furin. Immunohistochemistry was performed on heart tissue sections from three normal canines. The paraffin-embedded slides were deparaffinized. Immunocytochemistry was performed in HASMCs, which were fixed by 3% paraformaldehyde. A commercially available indirect immunoperoxidase kit (Vector Stain, Vector Laboratories, Burlingame, CA) was used as described previously (25). Briefly, after blocking was completed, tissues and cells were incubated overnight with a primary antibody for furin (1:200, Alexis) or corin (1:200, Abcam, Cambridge, MA). Cells were also incubated with a primary antibody for α-smooth muscle cell actin (1:500, Sigma-Aldrich, St. Louis, MO) as positive control. The sample incubated in nonimmune horse serum without primary antibody served as negative control. The specificity was further confirmed by substitution of nonimmune horse serum or PBS for primary antibody.

Quantitative RT-PCR. Total RNA was isolated from frozen tissue using the TRizol method, and the prepared RNA was reverse transcribed to synthesize cDNA. For quantitative comparison, real-time RT-PCR with Universal Probe Library hybridization monolayer probe sets was performed using a LightCycler480 System (Roche). Both negative and positive controls were included in each PCR reaction. All assays were performed as three independent PCR runs with 200 µg of each cDNA sample and normalized by hypoxanthine-guanine phosphoribosyltransferase gene expression, which is one of the stable housekeeping gene in canines (6). Sequences of primers are shown in Table 1.

Assays. Plasma renin activity, aldosterone, cGMP, ANP, BNP, and CNP levels were measured by radioimmunoassay as described previously (32). Tissue levels of ANP, BNP, and CNP were normalized for total protein concentration as determined by a bicinchoninic acid protein assay kit (Pierce, Rockford, IL).

Protein extraction from human and canine left ventricle tissues and Western immunoblot analysis. Total protein was extracted from commercially available LV in normal humans (ILSbio, Chestertown, MD), explanted LV tissue from a heart failure patient, canine LV or LA. For Western blot analysis, 30 µg of total protein were loaded onto polyacrylamide gels and transferred onto PVDF membranes. Membranes were blocked and incubated with a primary antibody against corin (1:1,000, Sigma-Aldrich) or furin (1:750, Alexis) overnight at 4°C. Membranes were washed in Tween-20 wash buffer and incubated with secondary antibody for 1 h at room temperature (RT). Bands were visualized by ECL Plus and exposure to X-ray film. Band intensity was quantified using the Fluor-S Multi Imager (Bio-Rad, Hercules, CA). The blots were stripped and reprobed with primary antibody for GAPDH (1:1,000, Novus Biologicals, Littleton, CO). Corin and furin protein expression normalized to GAPDH expression.

Statistical analysis. The primary analyses of the quantitative studies were expressed as means ± SE. Data between two groups were assessed by unpaired t-tests, in the comparison for normally distributed data, or Wisconsin rank sum test in the nonnormal distributed data. Nominal distribution of respective data was assessed by Shapiro-Wilk test. Statistical significance was accepted at P < 0.05.

RESULTS

Characteristics of experimental canine heart failure. Table 2 illustrates hemodynamic data and plasma neurohumoral factor

Table 1. Primer sequence of canine furin, corin, ANP, BNP, CNP, and HPRT mRNA

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>Furin</td>
<td>GCA ATA ATG GTC CCC ATG C</td>
<td>GCC TCC TCC TCC GAA TAT CC</td>
</tr>
<tr>
<td>Corin</td>
<td>TGC GCT ACG CAT CCC AGT C</td>
<td>CCC CTG CGA GAG AAG CAT</td>
</tr>
<tr>
<td>ANP</td>
<td>GAC GGT TCC TCC TCT TCT TGG</td>
<td>TGG GAC ACA GAG CGG TAC AC</td>
</tr>
<tr>
<td>BNP</td>
<td>GTC TCT GCA GCA CAT TGG</td>
<td>ACC CTG ACT TGT GGA TCA TGT</td>
</tr>
<tr>
<td>CNP</td>
<td>AGG GGC TTG GGA TGT TAG T</td>
<td>CAG CAA AAC GGA GGA AGT TC</td>
</tr>
<tr>
<td>HPRT</td>
<td>GCT CGA GAT TGT GCA AAG GAA</td>
<td>CCA TCA CTA TTT CTG TCC AGT GCT</td>
</tr>
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</table>

ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; HPRT, hypoxanthine-guanine phosphoribosyltransferase.
Corin and Furin in Experimental HF

Table 2. Characteristics of normal and heart failure canines

<table>
<thead>
<tr>
<th>Hemodynamic data</th>
<th>Normal (n = 4)</th>
<th>Heart Failure (n = 5)</th>
<th>P</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>133.4 ± 4.8</td>
<td>109.1 ± 5.2</td>
<td>0.0073</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>5.2 ± 0.6</td>
<td>21.1 ± 1.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>3.5 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>0.0027</td>
</tr>
<tr>
<td>SVR, mmHg·1⁻¹·min⁻¹</td>
<td>38.1 ± 2.1</td>
<td>47.3 ± 4.3</td>
<td>0.07</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>104.9 ± 6.0</td>
<td>240</td>
<td></td>
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</tbody>
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<tr>
<th>Neurohumoral factors</th>
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<tbody>
<tr>
<td>ANP, pg/ml</td>
<td>44.9 ± 18.4</td>
<td>468.1 ± 228.2</td>
<td>0.04</td>
</tr>
<tr>
<td>BNP, pg/ml</td>
<td>23.6 ± 10.5</td>
<td>40.3 ± 6.5</td>
<td>0.21</td>
</tr>
<tr>
<td>cGMP, pmol/l</td>
<td>7.5 ± 1.8</td>
<td>22.2 ± 2.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Angiotensin II, pg/ml</td>
<td>4.6 ± 0.64</td>
<td>28.0 ± 4.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Aldosterone, ng/dl</td>
<td>3.7 ± 0.7</td>
<td>9.0 ± 2.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Plasma renin activity, g·ml⁻¹·h⁻¹</td>
<td>1.3 ± 0.3</td>
<td>9.6 ± 1.8</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number canines. MAP, mean arterial pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; SVR, systemic vascular resistance; HR, heart rate; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide.

Levels of normal (n = 4) and heart failure (n = 5) canines. Pacing-induced heart failure was characterized by decreased CO and mean arterial pressure, increased PCWP, and a trend for higher systemic vascular resistance compared with normal canines. Circulating ANP, cGMP, angiotensin II, and plasma renin activity were significantly higher, and aldosterone tended to be higher in heart failure than in normal canines.

Next we confirmed corin and furin by Western immunoblot. Figure 2A shows representative staining for corin and furin in a normal canine heart. Immunohistochemistry for LA and LV tissues demonstrates positive staining for corin and furin in cardiomyocytes and vascular smooth muscle cells (Fig. 2A, arrows), suggesting the presence of both proprotein convertases in canine heart. Since corin expression in vascular smooth muscle cells has not been reported, we examined HASMCs. Positive immunohistochemical stainings for corin and furin were confirmed in HASMCs compared with negative control (Fig. 2B).

Detection of corin and furin protein in human and canine LV by Western immunoblot. Next we confirmed corin and furin protein were detectable by Western immunoblot for quantitative comparison in normal versus heart failure. On the Western immunoblot for corin, three different molecular mass bands were observed, ~150, ~130, and ~117 kDa proteins. The ~150- and ~130-kDa protein may represent N-glycosylated forms of corin that has suppressed enzymatic activity (14, 31). The predicted molecular mass of human (1042 amino acid) and canine corin (1030 amino acid) are 116 and 114 kDa, respectively, suggesting the ~117-kDa band is nonglycosylated corin (Fig. 3A). On the Western immunoblot for furin, two different molecular mass bands were observed, ~100 and ~75 kDa proteins. The ~100-kDa protein may be N-glycosylated profurin as already described by several groups (26, 28, 34), which may be an inactive precursor of furin. The predicted molecular masses of human and canine furin (680 amino acid) are 74 and 72 kDa, respectively, indicating the ~75-kDa band is nonglycosylated furin (Fig. 3A). Therefore, we used bands of nonglycosylated corin (~117 kDa) and furin (~75 kDa) for densitometric analyses for the comparison of normal versus heart failure tissue since they would be the active forms.

mRNA expression and protein concentration of natriuretic peptides in normal and heart failure canines. Quantitative RT-PCR demonstrated that ANP (Fig. 4A) and BNP (Fig. 4B) mRNA expression levels in normal LA were higher than those in normal LV. In contrast, CNP mRNA (Fig. 4C) showed no difference between LA and LV. Respective tissue assays for ANP (Fig. 4D), BNP (Fig. 4E), and CNP (Fig. 4F) revealed a similar pattern in normal LA versus normal LV.

ANP, BNP, and CNP mRNA expression levels in heart failure LA were higher than in normal LA but were not significantly different between heart failure LV and normal LV (Fig. 4, A–C). Respective tissue concentrations for ANP, BNP, and CNP revealed similar tissue concentrations for the natriuretic peptides, with markedly higher levels in heart failure LA but not in heart failure LV compared with normal (Fig. 4, D–F).

Levels of normal (n = 4) and heart failure (n = 5) canines. Pacing-induced heart failure was characterized by decreased CO and mean arterial pressure, increased PCWP, and a trend for higher systemic vascular resistance compared with normal canines. Circulating ANP, cGMP, angiotensin II, and plasma renin activity were significantly higher, and aldosterone tended to be higher in heart failure than in normal canines. Figure 1A illustrates representative picrosirius red staining for collagen protein content in canine LA and LV. The intensity of collagen staining increased dramatically in heart failure compared with normal, especially in LA (Fig. 1B), suggesting more remodeling occurred in LA than in LV by right ventricular pacing for 10 days.

Immunohistochemical and immunocytochemical staining for corin and furin in normal canine LA and LV and human aortic smooth muscle cells. Figure 2A shows representative staining for corin and furin in a normal canine heart. Immunohistochemistry for LA and LV tissues demonstrates positive staining for corin and furin in cardiomyocytes and vascular smooth muscle cells (Fig. 2A, arrows), suggesting the presence of both proprotein convertases in canine heart. Since corin expression in vascular smooth muscle cells has not been reported, we examined HASMCs. Positive immunohistochemical stainings for corin and furin were confirmed in HASMCs compared with negative control (Fig. 2B).

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mRNA and protein expression of corin and furin in normal and failing LA. As changes in natriuretic peptide gene expression and tissue levels where greatest in the atria with little changes in the ventricle, we defined mRNA and protein expressions of corin and furin in LA. Corin mRNA (Fig. 5A) and protein expression (Fig. 5B) in heart failure was lower in heart failure LA compared with normal LA. In contrast, furin mRNA expression in heart failure LA was higher than in normal LA (Fig. 5C), following the same pattern as natriuretic peptide expression in heart failure LA. Furin protein expression in heart failure trended to be higher than in normal LA but was not significant (Fig. 5D).

DISCUSSION

The current study confirms the important endocrine role of the atria in normal and in the early failing heart with respect to the natriuretic peptide system. Importantly, we observed as previously reported, marked activation of the natriuretic peptides in the atria. In accordance with the goal of our study, our investigation revealed that the key proproteins convertase corin was suppressed in the atrial myocardium even after only 10 days of experimental heart failure while furin was not. This reduction in corin gene and protein levels may have functional significance in contributing to altered natriuretic peptide molecular forms with reduced biological actions.

Our model of experimental heart failure was characterized by increased production of natriuretic peptides in the atria in association with widespread atrial fibrosis, which was not observed in the ventricle, underscoring the endocrine role of the atria. Previously, Luchner et al. from our group and Del Ry et al. reported temporal changes in BNP and CNP gene expression in pacing-induced heart failure, respectively (12, 33). Each paper respectively demonstrated that BNP or CNP production was dominant in the atrium compared with the ventricle. Interestingly, BNP expression in LV did not change while atrial BNP gene expression increased in early stage heart failure.

Fig. 2. Immunohistochemical staining for corin and furin in normal LV and LA, myocardium and vasculature (A), and immunocytochemical staining for corin and furin in human aortic smooth muscle cells (B). A: representative staining for corin (top), furin (middle), and negative control (bottom) in canine heart stained with/without polyclonal antifurin or anti-corin antibody. Arrows in tissues of vasculature represents positive stainings. Original magnification is ×40. B: representative staining for corin, furin, α-smooth muscle cell actin (α-SMA) and negative control in human aortic smooth muscle cells. Original magnification is ×100.
failure produced by 10 days of pacing as was observed in the current study, followed by both circulating BNP and BNP expression in LV markedly increasing in overt heart failure in which dogs were paced for 38 days (33). This early stage model of heart failure was a recent topic by the American Heart Association that stated the importance of developing animal models to address the unmet need of early stage heart failure models to understand the biology and to establish novel treatments to prevent the progression of heart failure (21). Here we reconfirm and extend those separate observations to include the selective increase in ANP and CNP gene expressions, complementing the increase in BNP gene expression, as well as the differential regulation of their respective proprotein convertases and strengthen these observations by including increases in protein concentrations.

In the current study we also examined normal and heart failure LA and LV tissue for fibrosis. We were surprised to see that collagen deposition in the normal LA was higher than in normal LV. This observation is however consistent with the seminal study of Oken and Boucek (35), which reported that the normal human atrium has a higher percentage of collagen compared with the ventricular myocardium in which muscle predominates thus explaining the greater distensibility of the atria compared with ventricular myocardium. Furthermore, after 10 days pacing, the collagen deposition was seen in both LA and LV, with greater fibrosis in the LA compared with the LV, suggesting the LA may be more sensitive to pro-fibrotic stimuli such as increased circulating angiotensin II, aldosterone, and renin levels (Table 2). This difference between atria and ventricle fibrotic response important in understanding the difference between the prevalence of atrial fibrillation (0.3% in overall population) (15) and ventricular tachycardia/sudden cardiac death (0–4% and 0.06–0.08%, respectively) in the United States population (1, 27).

Fig. 3. Western immunoblot for corin and furin protein in human and canine LV tissues. Representative blot for corin (A) and furin (B). N, normal (21 yr, male); HF, heart failure (48 yr, male, dilated cardiomyopathy); Ca, normal canine.

Fig. 4. mRNA expression and protein concentration of natriuretic peptides in normal and heart failure LV and LA. mRNA expression of ANP (A), BNP (B), and CNP (C) and protein concentration of ANP (D), BNP (E), and CNP (F) in normal (n = 4) and heart failure (n = 5) heart are shown. Values are means ± SE. Data are assessed by unpaired t-test except LV in BNP and CNP mRNA and ANP protein concentration, which are assessed by Wisconsin rank sum test since they are not normally distributed.

Data are assessed by unpaired t-test except LV in BNP and CNP mRNA and ANP protein concentration, which are assessed by Wisconsin rank sum test since they are not normally distributed.
How does the natriuretic peptide system influence atrial fibrosis? The increased fibrosis in the atria that occurred despite an increase in natriuretic peptide production may mean that mechanical stretch is more powerful in stimulating fibrosis in the atria than the anti-fibrotic actions of the natriuretic peptides. While studies have demonstrated in vitro and in vivo the prevention of fibrosis by the natriuretic peptides, it has not established whether natriuretic peptide system can reverse cardiac fibrosis in clinical settings. Therefore, caution is recommended when speculating a therapeutic role for the natriuretic peptides in reversing atrial fibrosis in heart failure and clearly further studies are needed.

We recently reported corin protein expression in normal human ventricle; however (23), the current study goes beyond previous reports and for the first time documents corin and furin protein localization in normal canine hearts in both atrium and ventricle as well as in vascular smooth muscle cells. These findings suggest corin/furin can process pronatriuretic peptides not only in the myocardium but also in vasculature structures, which may give important insights into the potential action of mature natriuretic peptide processing and concentrations that may affect circulating corin levels, in turn impairing fibroblast function but of vascular wall function as well.

We assessed corin and furin gene and protein expression for changes in normal versus heart failure LA, where we saw the greatest changes in natriuretic peptide expression. Previous studies have reported corin or furin gene and/or protein expression in the heart of human or rodent models (9, 29, 39). In our model, we found corin gene and protein expression decreased in heart failure LA. Similarly, Langenickel et al. (29) reported that corin mRNA expression decreased in LA of an infrarenal aortocaval shunt model of heart failure. Contrary to our findings, Tran et al. (44) reported that both ANP and corin mRNA increased in LV tissue of a rat heart failure model of myocardial infarction, and in a human study, corin protein expression in end-stage heart failure LV tissue increased compared with those in nonfailing hearts (9). These findings suggest that corin expression may differ in disease states, with severity/duration, and importantly, be differentially regulated in the atrium versus the ventricle. Dong et al. (13) reported that circulating soluble corin levels, where corin is shed from the cell membrane, decreased in patients with heart failure (24). In the current study, corin expression in failing atrium decreased, which may affect circulating corin levels, in turn impairing pro-ANP and pro-BNP processing in the atrium as well as in the circulation. The impairment of pro-ANP/BNP processing could contribute atrial fibrosis since mature ANP and BNP have anti-fibrotic effects through GC-A.

Examining furin expression in our model, we report furin mRNA expression significantly increased, following the natriuretic peptide expression pattern (Fig. 5, C and D) with induction of heart failure. Similarly, Sawada et al. (39) reported that furin mRNA expression was coelevated with BNP mRNA in both the atrium and ventricle in the myocardial infarcted rat. Indeed, the elevation of furin may have implications beyond the natriuretic peptides as furin can process not only pronatriuretic peptides but also pro-transforming growth factor (TGF)-β1, pro-endothelin-1, and pro-MT-matrix metalloproteinase-1 (MMP1), which are activated in heart failure (37, 41, 46). Specifically, TGF-β1 induces MMP-1 activity and amplifies furin, which is also required for MT1-MMP/MMP2 activation in vitro, suggesting furin may play a central role for in TGF-β1/MMPs-related remodeling (42). In our current study, furin mRNA and protein expression was coelevated with natriuretic peptides and fibrotic changes in LA, suggesting furin may play a role in not only extracellular matrix protein-
related remodeling but also in pro-BNP and pro-CNP processing to mature BNP and CNP with differential regulation compared with corin.

Why are corin and furin differentially regulated in the failing LA? To date, there are no reports on neurohumoral/cytokine activation or suppression of corin production in either normal or pathological states. Bando et al. and Blancette et al. reported that TGF-β1 stimulated furin production (3, 4); however, the regulation of both enzymes is likely to be multifactorial, such as mechanical and neurohumoral/cytokine mechanisms with negative/positive feedback loops. Additional studies are needed to understand what regulates corin and furin expressions especially in heart failure, whether the expression of one affects the expression of the other, and what role each has in disease progression.

Further studies should be performed to clarify whether pronatriuretic peptides processing is reduced especially for pro-ANP and pro-BNP. Our current assays for natriuretic peptides protein concentration are nonspecific and may detect not only mature natriuretic peptides but also pronatriuretic peptides, which is a limitation to our current study. Further analysis is needed using more specific assays and mass spectrometry.

**Perspectives and Significance**

The current study has three clinical implications. First, atrial remodeling may be an important area of focus in early stage heart failure both from a diagnostic and therapeutic perspective. Importantly, Anter et al. (2) elegantly reviewed the association between atrial fibrillation and heart failure. Heart failure mediated atrial fibrosis and remodeling with atrial fibrillation occurred with worsening cardiac function and was associated with worsening outcomes in heart failure patients. Thus we need to develop therapeutic interventions in early stages of heart failure in preventing atrial fibrosis. Indeed, as furin levels are elevated in the atria in early stage disease, it could serve as a biomarker for atrial fibrosis and, second, recognizing the potent anti-fibrotic actions of CNP perhaps pro-CNP could serve as a potential anti-fibrotic drug targeting the atria also recognizing the increase in furin expression. Second, our model is a potential model to understand not only the necessity potential anti-fibrotic therapy against atrial remodeling but also in understanding corin/furin and natriuretic peptide system regulation in early stage heart failure. Decreased corin expression in the atrium may indirectly have contributed to atrial fibrosis with less conversion of pro-ANP or pro-BNP into ANP and BNP, reducing their anti-fibrotic properties. Increased furin may enhance conversion of pro-CNP into CNP, however, it may also lead to an increase in mature pro-fibrotic proteins. Finally, GC-A agonists such as mature ANP and BNP, which are essential for the control of natriuresis, vasorelaxation, inhibition of renin and aldosterone, and suppression of fibrosis and myocyte hypertrophy, may be decreased in a model of early heart failure due to impaired processing by decreased corin production in atria. Indeed, this supports the use of ANP or BNP as therapeutics in heart failure or perhaps even corin itself to cleave pro-ANP or pro-BNP into their mature forms. Clearly, further studies are required to fully understand the therapeutic relevance of the current observations.

In conclusion, corin and furin are widely present in the normal heart but display opposing gene and protein expression patterns in the atrium of rapid pacing-induced heart failure, a model characterized by structural remodeling and activated natriuretic peptides production. Differentially regulated corin and furin may modulate pronatriuretic peptide conversion to active natriuretic peptides and therefore may influence the activity levels of natriuretic peptides in not only heart tissue but also in the circulation in heart failure. This interesting selective change of corin and furin may have significance in the regulation of natriuretic peptide processing and ultimately in cardiorenal function and therapeutic strategies in heart failure.

**GRANTS**

This work was supported by grants from the National Institute of Health (RO1 HL-36634 and PO1 HL-76611) awarded to J. C. Burnett Jr., American Heart Association Post-Doctoral Fellowship awarded to T. Ichiki (10PPOST3660045), American Heart Association Scientist Development Grant awarded to T. Ichiki (12SDG11460017), and the Mayo Foundation.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**